Docket No. 050992.0201.03USCP

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Itzhak Bentwich, *et al.*
App. No.:	10/708,204
Conf. No.:	2203
Filing Date:	February 16, 2004
Art Unit:	1635
Examiner:	WOLLENBERGER, LOUIS V
Title:	BIOINFORMATICALLY DETECTABLE GROUP OF NOVEL REGULATORY OLIGONUCLEOTIDES ASSOCIATED WITH ALZHEIMER'S DISEASE AND USES THEREOF

DECLARATION OF AYELET CHAJUT, PH.D.

Dear Sir:

I, Ayelet Chajut, Ph.D., hereby declare as follows:

1. I am the Executive Vice President, R&D at Rosetta Genomics, Ltd. ("Rosetta"). A true and correct copy of my Curriculum Vitae is attached to this declaration as Exhibit A.
2. I have 22 years of experience designing and performing experiments in the field of molecular biology, 2.5 of which were related to miRNA biology. I have also worked in the biotechnology industry for 10 years.
3. As a result of my work as Executive Vice President, R&D and experience in the field of molecular biology, I supervised and conducted the two sets of experiments described herein at items 4-7.
4. In order to confirm that the miRNA hsa-miR-151 is expressed in Hep3B cells, the following microarray experiments were performed. RNA from Hep3B cells was extracted and hybridized to a microarray. Signal detected from a microarray is normally spread in a certain range. The lower part of this range is usually below 300, and is considered to be a background level. The average expression signal (1556.250) was higher than background, therefore showing that hsa-miR-151 was expressed in Hep3B cells.
5. The results of the microarray experiments are summarized in the following table. The table includes the maximum, average, and standard deviation of hsa-miR-151

expression measurements in the Hep3B cell line and the number of sample measurements.

SourceName	miR name	Max_signal	Average	Std	Number
Hep3B2.1-7	hsa-miR-151	2203	1651.857	338.4733	7
Hep3B2.1-7	hsa-miR-151	3723	1504.769	710.5591	13

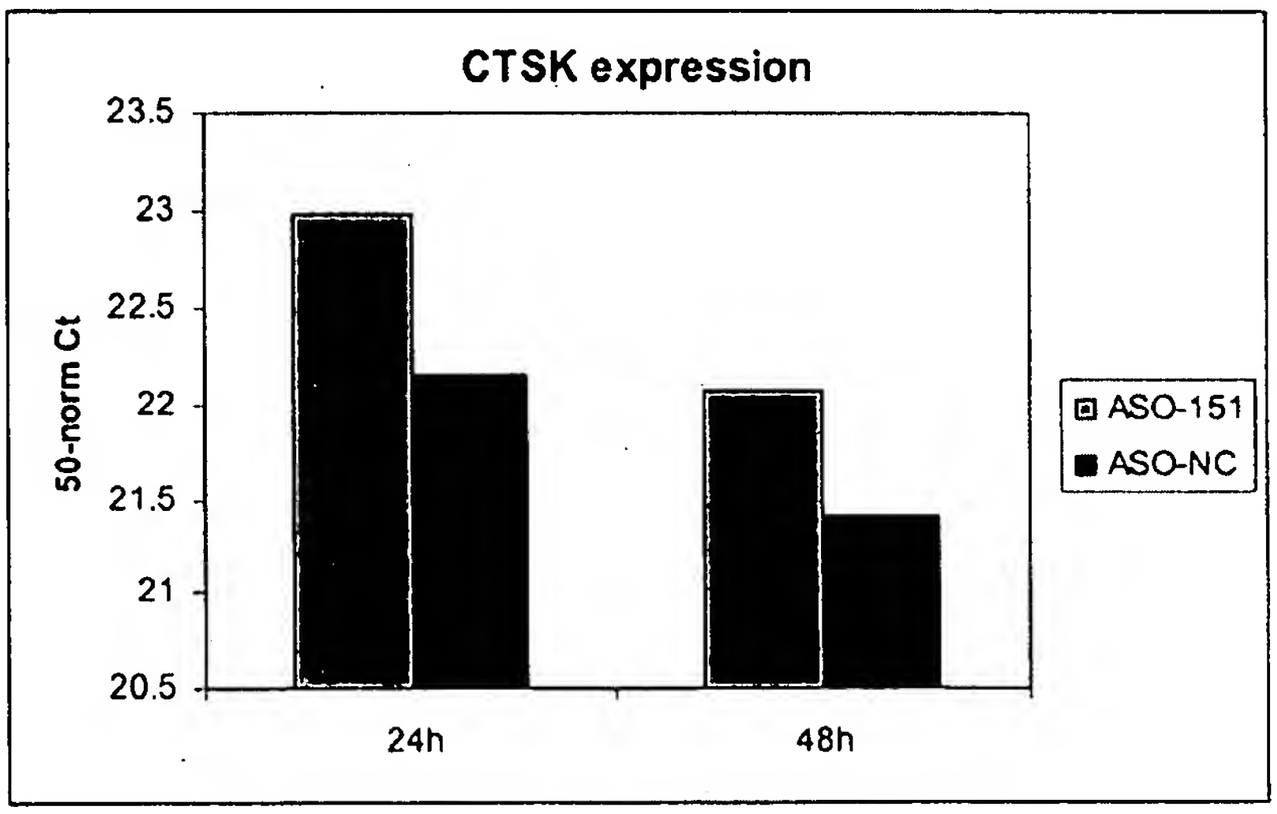
6. In order to confirm that the hsa-miR-151 affects mRNA levels of human cathepsin K preprotein ("CTSK"), Hep3B cells were transfected with an anti-sense oligonucleotide ("ASO") that specifically binds to hsa-miR-151, and the resulting levels of target human CTSK mRNA were measured and compared to the level of target in cells transfected with negative control ASO . Hep3B cells from the American Type Culture Collection (Rockville, MD) were plated in 10-cm tissue culture plates 24 hours prior to transfection. Cells were then transfected with the specific ASO to hsa-miR-151 for 24 hours using Oligofectamine (Invitrogen, Carlsbad, CA) . After transfection, RNA was isolated and human CTSK mRNA was quantified using the specific primers listed below by the SYBR quantitative reverse-transcription polymerase chain reaction ("qRT-PCR") method (Applied Biosystems). mRNAs of the housekeeping genes TBP and RPS20 were also quantified by SYBR qRT-PCR using the primers below.

			Primers for Target	
miR	Target	ASO for miR	Fwd	Rev
hsa-miR-151	CTSK	CCTCAAGGAGCTTCAGTCTAG	GTTTTGGCAGTGGGATATGG	CAGGCGTTGTTCTTATTTCG
house keeping	RPS20		AACAAGCCGCAACGTAAAAT	GGAAACGATCCCACGTCTTA
house keeping	TBP		TATAATCCCAAGCGGTTTGC	CACAGCTCCCCACCATATTC

Total RNA was isolated by EZ-RNA II kit (Biological Industries). 1μg of total RNA was reverse transcribed using Supercript II. After reverse transcription, 10ng of cDNA were used in a qRT-PCR reaction. mRNA was quantified by qRT-PCR SYBR Green method (Applied Biosystems) using a 7500 Fast Real Time PCR system. Each test was done in triplicate. Measuring the amount of initial mRNA was based on the observation that the amount of cDNA generated from the mRNA doubles with every cycle of PCR. Therefore, after N cycles, there is 2^N times as much. The initial amount of mRNA was quantified by measuring the cycle number at which the increase in fluorescence (and thus the amount of cDNA) was

exponential. A threshold at this level of fluorescence was set. The cycle at this point is indicated as the cycle threshold, or Ct. To compare the differences in quantity between a specific mRNA in two different samples, the 50-Ct value was calculated from the Ct value for each of the samples, and the delta 50-Ct (d50-Ct) was calculated. The fold-change between the amount of mRNA in the two samples was represented by $2^{d50\text{-}Ct}$ (or 2^{dCt}). Normalization was done by subtracting the Ct values of the housekeeping genes TBP and RPS20. Ct values were determined using a default threshold of 0.2 in the 7500 Fast Real time PCR system (Applied Biosytems), and Ct values were normalized to the housekeeping genes TBP and RPS20.

7. Affects of the hsa-miR-151 ASO on human CTSK expression in Hep3B cells are shown below:



The above plot shows that the level of human CTSK mRNA in cells transfected with an ASO that inhibits hsa-miR-151 is increased approximately 1.8-fold (*i.e.*, $2^{d50\text{-}Ct}=2^{(22.95\text{-}22.10)}$) at 24 hours and 1.6-fold (*i.e.*, $2^{d50\text{-}Ct}=2^{(22.10\text{-}21.40)}$) at 48 hours as compared to non-ASO control cells.

8. I solemnly declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18

U.S.C. § 1001, and may jeopardize the validity of the application or any patent issuing thereon.

Dated: 19, May 2009 By: A. Chajut
Ayelet Chajut, Ph.D.